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A detailed comparison of the 5'-end of the ovalbumin gene cloned from chicken oviduct and erythrocyte DNA

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ABSTRACT

We have examined homologous fragments of DNA cloned from two different tissues for changes in the DNA sequence which might be related to tissue specific gene expression. The 5' end of the chicken ovalbumin gene was cloned from oviduct or erythrocyte DNA using cosmids as vectors. We have compared the two clones obtained by restriction enzyme digestions, analysis of heteroduplexes by electron microscopy or S1 nuclease digestion and by DNA sequencing. Our results show that whereas no alteration occured in the region of the gene assumed to be of importance for the control of transcription, a 4 nucleotide deletion/insertion was detected in the first intron of the ovalbumin gene.

INTRODUCTION

It is widely accepted that DNA is a stable structure invariant from cell to cell in a given organism (except for random mutational events), in keeping with its central role as the source of genetic information which defines a species. However, there is ample evidence that variations in the DNA sequence can occur within an organism under a variety of different circumstances. These changes can be broadly classified as follows : (i) rearrangements (1,2) ; for example the immunoglobulin genes [see review of Dunnick (1), for references]. (ii) amplifications (3-9) ; for example the mouse dihydrofolate reductase gene (3). (iii) transpositions (10-14) ; for example the genes which confer different mating types on yeast,[see review of Leupold (10) for references] and (iv) inversions (15-17); at present described only for prokaryotes, for example the flagellin gene of salmonella(15).

In view of these examples it is important to consider the possibility that DNA rearrangements could be a general mechanism involved in the regulation of gene expression in eukaryotes. The

examples cited above involve, in most cases, fairly radical rearrangements of nucleotide sequences in the genome which have been detected by the hybridization of cellular DNA, digested with restriction enzymes, with probes for the genes analysed. It is possible also that more subtle changes occur in the primary structure of a gene. As a result of recombinant DNA technology it is now feasible to obtain large quantities of eukaryotic genes purified from different tissues and to undertake an analysis of such alterations. The experiments reported in this paper are a first attempt to examine this question of general biological importance.

In the oviduct of the laying hen approximately 50% of the mRNA is ovalbumin mRNA (ov-mRNA) whereas in other tissues Ov-mRNA is a very minor component of the mRNA (18) and in chicken erythrocytes it is undetectable (19). The large amount of Ov-mRNA produced in the oviduct is not due to gene amplification in that tissue (20,21) and seems to be adequately accounted for by increased transcription and stability of Ov-mRNA (22). However, it appears possible, *a priori*, that changes in the DNA sequence of the ovalbumin gene could also increase its efficiency of transcription in the oviduct compared to other tissues.

We,and others have previously established the structure of the chicken ovalbumin gene in cloned erythrocyte DNA (see ref. 23 for references). In addition we have shown that total DNA prepared from a variety of chicken tissues is indistinguishable when hybridized with purified ovalbumin probes after digestion by several restriction enzymes (24,25), with the exception of changes due to the DNA methylation pattern (26) or minor allelic variations (27,28). For a more detailed analysis, we now compare the structure of the 5'-end of the ovalbumin gene cloned from chicken erythrocyte and chicken oviduct DNA. The 5'-end was chosen for this study as it is the region involved in the initiation of transcription and therefore is mostlikely to have an influence on the control of the expression of the gene. In this paper we present the results of a comparison of restriction enzyme digest patterns, of electron microscopic analysis, of the action of S1 nuclease on DNA heteroduplexes and finally DNA sequence data for the DNA cloned from the two tissues.

MATERIALS AND METHODS

Cloning.

The DNA fragment Eco6-Eco7 (see Fig. 1b) had previously been cloned from chicken erythrocyte DNA using a cosmid vector by Royal et al. (29). To clone the corresponding fragment from the oviduct, chicken oviduct DNA (from a different chicken) was purified by the method of Bellard et al. (30), digested to completion by the restriction enzyme EcoRI and fragments of approximately 15 kilobases (kb) were selected after a sucrose gradient. 2 µg of this DNA was ligated in a volume of 20 µl with 6 µg of the cosmid pJC 75.58 (31) and used to transduce the *E.coli* strain 803 r_k^- m_k^- SupE SupF (32) using the packaging method and strains of bacteria described by Hohn (33). Transformed bacteria were selected by their growth on ampicillin plates and those which contained the 14 kb 5' terminal ovalbumin EcoRI fragment Eco6-Eco7 (see Fig. 1b) were selected after *in situ* hybridization with the probe Pst3-Pst4 (see Fig. 1b). The erythrocyte and oviduct Eco6-Eco7 fragments of DNA, were subcloned into the EcoRI site of pBR322 [pBR(Eco6-Eco7)Er and pBR(Eco6-Eco7)Ov] , and subsequently the Eco6-Pst4 fragments (see Fig. 1b) were subcloned into the corresponding unique sites of pBR322, [pBR(Eco6-Pst4)Er and pBR(Eco6-Pst4)Ov]. Growth and preparation of cosmid and plasmid DNA were essentially by the cleared lysate technique of Clewell and Helinski (34) followed by ethidium bromide-CsCl equilibrium gradients.

Heteroduplex analysis by electron microscopy.

The 14 kb EcoRI fragment from the erythrocyte (Eco6-Eco7) (see Fig. 1b) was purified on a sucrose gradient after digestion of the plasmid pBR (Eco6-Eco7)Er with the restriction enzyme EcoRI. The plasmid pBR (Eco6-Eco7)Ov was linearized by the restriction enzyme SalI. To form a heteroduplex between the two species, 1-3 µg/ml of each DNA were mixed together and denatured by heating for 5 min at 75°C in 70% deionized formamide, 300 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA pH 8.5. The samples were then reannealed for 30 min at 25°C before mounting for electron microscopy as previously described (25).

S1 nuclease mapping of DNA heteroduplexes.

To probe for differences in the sequences of linearised pBR (Eco6-Pst4)Er and pBR(Eco6-Pst4)Ov, the two DNAs were mixed at equal concentrations to give a final total concentration of 2-2.5 µg/ml in 500 µl of 0.1 M NaOH. After incubation for 10 minutes at 20°C the mixture was carefully neutralised to pH 7 by the addition of 1 M HCl. NaCl was added to give a final concentration of 250 mM and the sample was allowed to reanneal at 60°C for 1 h. The S1 nuclease treatment, which followed immediately, was in 30 mM Na CH_3COOH pH 4.5 4 mM Zn Cl_2 and 230 mM NaCl (final concentration) with 150 units of nuclease S1 at 37°C for the times indicated in the legends to figures 4 and 5. The reaction was stopped for each time point by adding 60 µl of the sample to a 500 µl Eppendorf conical tube which contained (final concentration) Tris-HCl 60 mM pH 7.5, EDTA 6 mM, SDS 1% at 4°C. At the end of the time course the volume in each tube was increased to 120µl by addition of Tris-HCl 10 mM pH 7.5, EDTA 1 mM. Phenol (60 µl saturated by Tris-HCl 50 mM pH 8) was added and, after vortexing, 60 µl of chloroform were added. After centrifugation, the aqueous phase was made 200 mM in NaCl and precipitated by 2 volumes of ethanol by repeated freezing(in liquid nitrogen) and thawing. After centrifugation and lyophilization to remove any residual ethanol, the samples were dissolved in 30 mM NaOH, 2 mM EDTA. Electrophoresis was performed at room temperature using the alkaline gel method described by McDonnel et al. (35) on 1.2% agarose alkaline gels. After electrophoresis, the gels were placed in Tris 50 mM acetate 20 mM pH 7.9, EDTA 1 mM, and stained with ethidium bromide for photography of DNA size marker bands. The DNA in the gels was then transferred to nitrocellulose filters as described (36) using the method of Southern (37). Subsequently the gels were hybridized with [^{32}P]-labelled nick-translated (38) pBR (Eco6-Pst4)Er or Ov (Fig. 1c) washed (36) and subjected to autoradiography (36). The sizes of the DNA bands which hybridized were estimated by comparison with the UV markers. To control the action of S1 nuclease on homoduplexes the experiment was performed as above except that the pBR (Eco6-Pst4)Er and pBR (Eco6-Pst4)Ov were denatured and reannealed separately and were then mixed before the addition of S1 nuclease.

DNA sequencing.

The plasmids pBR (Eco6-Pst4)Er and Ov were digested by the restriction enzyme HindIII which cuts the DNA once in the pBR322 sequence (39) and once in the (Eco6-Pst4) fragment (see Fig. 1c). After end-labelling with [^{32}P]-γ-ATP as in reference (40), the DNA was digested by the restriction enzyme EcoRI (see Fig. 1c) and the fragments HindIII-Eco6 Ov and Er were purified from a 5 % acrylamide gel. The chemical degradation method of Maxam and Gilbert (41) was applied to the fragments before electrophoresis on denaturing 10% polyacrylamide 8.3 M urea gels (41, 42).

Materials.

The restriction enzymes EcoRI and HindIII were prepared according to the procedures of Sumegi et al. (43) and Humphries et al. (44). Other restriction enzymes were purchased from New England Biolabs or Bethesda Research Laboratories. Restriction enzyme digests were performed as described in the New England Biolabs booklet. The S1 nuclease was purchased from Bethesda Research Laboratories and was diluted before use in the buffer which they recommend. The cosmid pJC 75.58 was a gift from John Collins.

RESULTS

Cloning of the 5'-end of the ovalbumin gene from oviduct DNA.

We have used cosmids (33,45) as vectors for the cloning from oviduct DNA of a 14 kb EcoRI fragment (Eco6-Eco7) which is located at the 5'-end of the ovalbumin gene (see Methods and Fig. 1b). This fragment contains a portion of the first intron of the ovalbumin gene, the leader exon, the presumed ovalbumin promoter region and the 3'-end of the Y gene which contains some exonic sequences which are homologous to the ovalbumin gene (23,29,46, and Heilig, R., Muraskowski, R. and Mandel, J.L., personal communication). A detailed characterisation of this region of the chicken genome has previously been performed on the analogous fragment of the ovalbumin gene cloned from erythrocyte DNA by Royal et al. (29), see also (23) and (46). The experiments

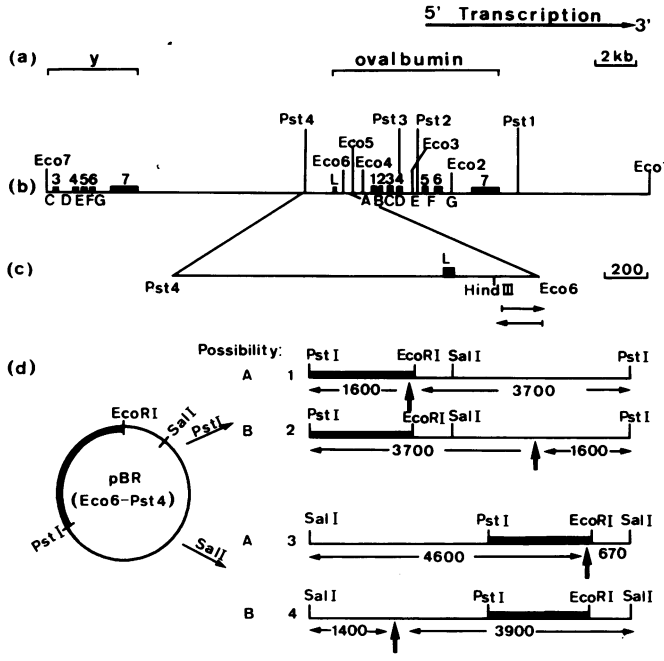


Fig. 1 : (a) Localisation of the chicken ovalbumin gene and part of the Y gene, scale in kilobases (kb) for (b), and the direction of transcription. (b) Localisation of the exons (heavy lines, L, 1-7) and introns (A-G) in the ovalbumin and part of the Y gene (Refs. 23, 29, 46 and Heilig, R., Muraskowski, R. and Mandel, JL, personal communication). The Eco and Pst sites correspond to the EcoRI and PstI restriction enzyme sites which were previously mapped (see references in ref. 23). (c) The Eco6-Pst4 fragment which was used for the S1 nuclease and DNA sequencing experiments. The arrows show the direction and the extent of the sequence determination from the HindIII and EcoRI sites. (d) A representation of the possibilities for the location of the site of the mismatch detected by the S1 nuclease in the heteroduplexes formed between the linearized pBR(Eco6-Pst4)Er and Ov plasmids (see Fig. 4 and 5). The plasmid is represented as a circle with the heavy line corresponding to the ovalbumin Eco6-Pst4 fragment. After linearization with the restriction enzyme PstI (lines 1 and 2) and heteroduplex formation (see Methods), the S1 nuclease detected a mismatch in the sequence of approximately 1600 bp from the extremity of the linear plasmids (see Fig. 4). The two possibilities for the location of this mismatch (A and B) are indicated by the heavy vertical arrows. Lines (3) and (4) show, for each of these two possibilities, the predicted location of this mismatch relative to the extremities generated by the digestion of the plasmid by the restriction enzyme SalI. The lengths of the DNA fragments generated (1 and 2) or predicted (3 and 4) are given in base pairs (for clarity, the lengths of the fragments in line 3 are those found in the experiment shown in Fig. 5).

described below were performed on either the entire 14 kb EcoRI fragments or on the Eco6-Pst4 fragments, (see Fig. 1c) which contain the ovalbumin promoter region (23,47),which were sub-cloned in the homologous sites of the plasmid pBR322.

Comparison of patterns of restriction enzyme digestion.

The two plasmids pBR (Eco6-Eco7)Er and pBR (Eco6-Eco7) Ov (see Methods) were digested with EcoRI plus 16 different restriction enzymes. The digested DNA was electrophoresed on agarose gels and photographed after visualisation of the DNA bands by ethidium bromide. The comparison of these digestion patterns (some of which are shown in Fig. 2) shows that for most enzymes no difference could be detected between the DNA cloned from the two tissues. However, with some enzymes (see Fig. 2, lanes B and b) minor changes in the band patterns were observed (see arrow). A more detailed analysis was therefore required,

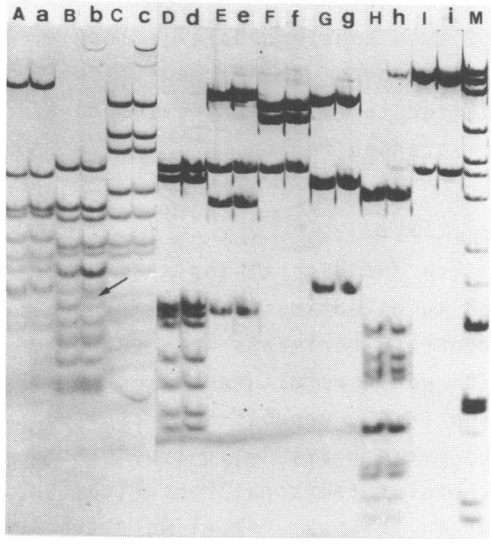


Fig. 2 : Comparison of patterns of restriction enzyme digestions. The DNA of the plasmid pBR (Eco6-Eco7)Ov (capital letters) or pBR (Eco6-Eco7) Er (small letters), was digested by the restriction enzyme EcoRI (lanes I and i) or EcoRI plus HinfI (A and a), Sau96 (Band b), AluI (C and c), BglII (D and d), XbaI (E and e), KpnI (F and f), BamHI (G and g) and PstI (H and h). The DNA was electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. Lane M contains DNA size markers (a mixture of Adenovirus-2 DNA digested by EcoRI and BamHI and SV40 DNA digested by HindIII). The sizes of the fragments in lane M are (in kilobases, starting from the top of the gel) : 21.05, 14.6, 10.4, 6.3, 4.7, 4.4., 3.7, 2.9, 2.05, 1.77, 1.15, 1.1, 0.52 and 0.45. Extra bands at the top of some lanes are due to incomplete digestions. The arrow points to a difference in the pattern of DNA digestion between lanes B and b.

since the change might reflect a small region of rearrangement or simply allelic variability at some restriction sites as previously observed (24,27,28).

Electron microscopic analysis of heteroduplexes.

A more sensitive comparison of the DNA from both tissues was performed by the examination of heteroduplexes by electron microscopy. This method could reveal a mismatch of 50-80 base pairs (bp) in a DNA-DNA heteroduplex (F.P., unpublished results). For this study it was important to distinguish between homoduplexes and heteroduplexes which theoretically are formed in equal amounts when the two DNAs are mixed, denatured and reannealed (see Methods). To this end, the heteroduplex was formed between the purified 14 kb erythrocyte fragment and the linearized plasmid [pBR (Eco6-Eco7)Ov] which contained the homologous fragment cloned from oviduct DNA (see Methods). When a heteroduplex is formed between those two molecules one expects a 14 kb double stranded portion straddeled by 2 single-stranded arms of 3.65 and 0.65 kb length. An electron micrograph of such a heteroduplex molecule is shown in Fig. 3. Careful examination, at higher magnification, of this, and other molecules, failed to reveal any evidence of mismatching in the 14 kb double-stranded region.

S1 nuclease mapping of heteroduplexes.

S1 nuclease can recognise and digest single-stranded DNA and has previously been used to detect mismatches in DNA heteroduplexes (48, 49). Preliminary experiments were performed to determine the conditions which would permit the detection by S1 nuclease of a mismatch of 9 bp (F.G., unpublished results) present in a heteroduplex formed between SV40"wild-type" and a deletion mutant prepared by C. Benoist (personal communication). The factors which were important in this experiment were (a) the absence of nicking activities in the restriction enzyme used to linearize the plasmids, (b) a low concentration of DNA (2-2.5 µg / ml when the plasmids were denatured (c) a very active S1 nuclease (d) the salt concentrations and (e) the temperature.

Using the conditions described in the Methods section, we

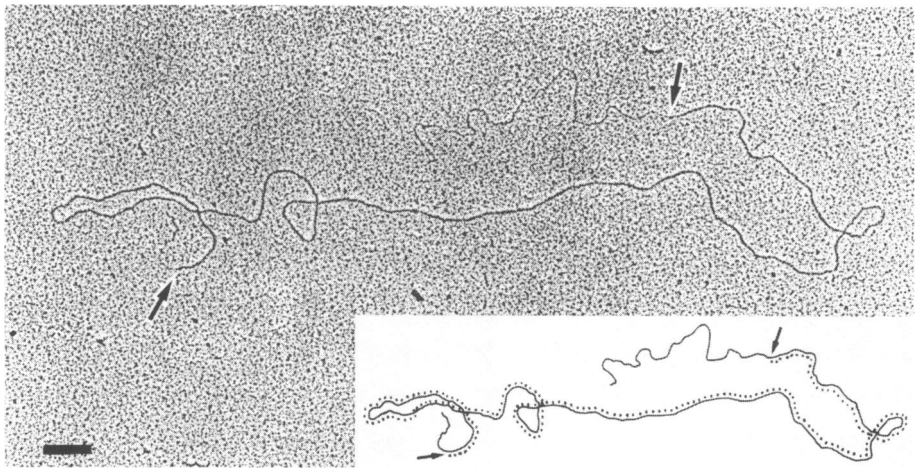


Fig. 3 : Electron microscopic analysis of heteroduplexes. Heteroduplexes were formed (as described in Methods) between the purified 14 kb Eco6-Eco7 erythrocyte DNA fragment (dotted line on the line drawing) and the plasmid pBR (Eco6-Eco7)Ov which was linearized by digestion with the restriction enzyme SalI (full line on the line drawing). The arrows indicate the limits of the double-stranded portion of the heteroduplex.From the measurements of 15 molecules, the length of the double-stranded hybrid is 14244 ± 222 bp. The lengths of the single-stranded (pBR) arms are 641 ± 62 bp and 3451 ± 84 bp. The line bar represents 0.1 μm.

probed the heteroduplexes formed between erythrocyte and oviduct DNA for mismatches. Initial experiments with the 14 kb Eco6-Eco7 fragment proved inconclusive due to random nicking of the DNA under the conditions chosen. We therefore decided to restrict our analysis by S1 nuclease to the Eco6-Pst4 fragment which contains 350 bp of intron A, the 45 bp leader exon (L) and about 1,300 bp 5' upstream from the first nucleotide of the leader exon (23, see Fig. 1b). The plasmids pBR (Eco6-Pst4)Er and pBR (Eco6-Pst4) Ov were linearized at the Pst site (see Fig. 1b). They were then mixed, denatured and reannealed to form heteroduplexes (and inevitably, homoduplexes) and treated with S1 nuclease, as described in the Methods section. As shown in Fig. 4, two distinct new bands (indicated by arrows) were formed when the heteroduplexes (Lane 4) were probed by S1 nuclease (in addition to the unaltered DNA

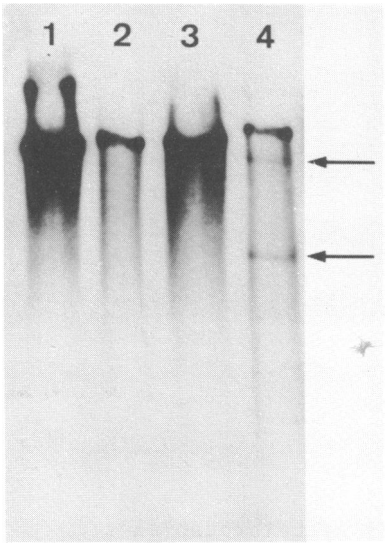


Fig. 4 : The action of S1 nuclease on DNA heteroduplexes. The plasmids pBR (Eco6-Pst4)Er and pBR (Eco6-Pst4)Ov were linearized with the restriction enzyme PstI. For lanes 1 and 2, the oviduct and erythrocyte pBR (Eco6-Pst4) DNAs were denatured, and reannealed separately and then mixed before incubation for 15 minutes with (lane 2) or without (lane 1) S1 nuclease. For lanes 3 and 4, the oviduct and erythrocyte pBR (Eco6-Pst4) DNAs were mixed together before denaturation, reannealing and incubation for 15 minutes with (lane 4) or without (lane 3) S1 nuclease (see Methods). Subsequently the DNA was electrophoresed on a 1.2% agarose-alkaline gel, transferred to a nitrocellulose filter and hybridized with a [^{32}P]-nick-translated pBR (Eco6-Pst4) probe (see Methods). After washing, the DNA bands which hybridized were revealed by autoradiography. The arrows indicate the positions of the extra bands detected after S1 nuclease digestion of the heteroduplex (lane 4). Lanes 1 and 3 were overloaded with DNA to show that the extra bands of DNA found in lane 4 were due to the action of S1 nuclease.

band due to the homoduplexes present) whereas none are found when the mixture of homoduplexes only were digested (Fig. 4, lane 2). Incubation for longer times (up to 1 h) or of the samples 5-fold more concentrated in DNA revealed no extra bands (data not shown). By comparison with DNA size markers (see methods), the sizes of the fragments found after S1 nuclease treatment of the pBR (Eco6-Pst4) heteroduplex are 1,600 and 3,700 bp. The mismatch detected by S1 nuclease is therefore either close to the EcoRI site 6 or at the other extremity of the linearized molecule within the pBR322 plasmid sequences (see Fig. 1d, 1 and 2).

To distinguish between these two possibilities (indicated A and B respectively in Fig. 1d) we linearized the pBR (Eco6-Pst4) molecules with the restriction enzyme SalI and repeated the S1 nuclease treatment of the heteroduplex molecules. This experiment confirmed that the S1 nuclease detected a non-hybridizing region in the heteroduplex. Fig. 5A shows (lanes 5 and 6, indicated by arrow) that the mismatch occured very close to the extremity of

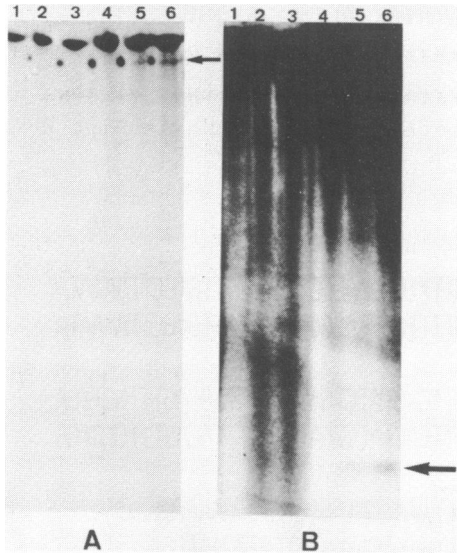


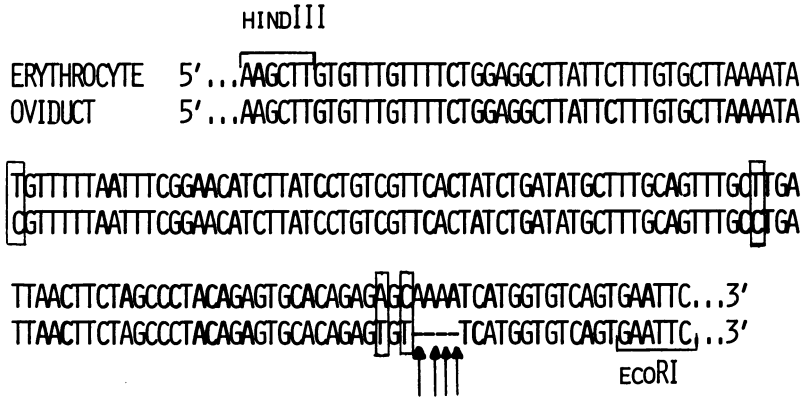
Fig. 5 : The action of S1 nuclease on DNA heteroduplexes. As in the legend for Fig. 4, except that the plasmids pBR (Eco6-Pst4)Er and Ov were linearized by the restriction enzyme SalI. Lanes 1-3 contain only homoduplexes and lanes 4-6 contain a mixture of homoduplexes and heteroduplexes (see legend to Fig. 4). Lanes 1 and 4 were incubated in the absence of S1 nuclease. Lanes 2 and 5 were incubated with S1 nuclease for 15 min. and lanes 3 and 6 for 60 min. After electrophoresis, transfer to nitrocellulose filters and hybridization with the [^{32}P]-labelled pBR (Eco6-Pst4) probe, the DNA bands which hybridized were revealed by autoradiography for 6 hours (A) or 48 hours (B). The arrows indicate the new bands of DNA detected as a result of the action of S1 nuclease.

the molecule, in keeping with possibility A (see Fig. 1c). Overexposure of the autoradiograph (Fig. 5B) revealed a second fragment (indicated by an arrow) of 670 bp which was formed by S1 nuclease digestion of the heteroduplex (see Fig. 5B, lanes 5 and 6 and Fig. 1c). This result, in conjunction with the S1 nuclease digestion of the PstI linearized molecules places the mismatch between the two plasmids within the ovalbumin Eco6-Pst4 fragment at approximately 20 bp from the EcoRI site (see Fig. 1c, lines 1 and 3).

Sequence studies.

To confirm the result of S1 nuclease mapping, to locate this mismatch precisely and to determine its extent, we sequenced the region of the [Eco6-Pst4]Er and Ov fragments (see Fig. 1c for the sequencing strategy) at which the S1 nuclease digested the heteroduplex. There is one HindIII site in this fragment and another in pBR322 at 29 bp from the site of EcoRI (39). To sequence the region HindIII-Eco6 of the Eco6-Pst4 fragments (see Fig. 1c) we digested pBR (Eco6-Pst4)Ov

or Er with the restriction enzyme HindIII, 5'-end labelled with [^{32}P]-γ-ATP (as in ref. 40) and then digested the HindIII fragment with the restriction enzyme EcoRI which cuts at the site of Eco6. The sequence and sequencing gel shown in Fig. 6



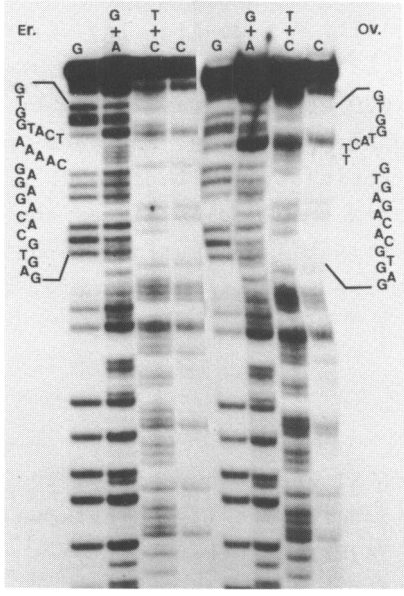


Fig. 6 : Comparison of the nucleotide sequence of the Hind III-Eco6 (see Fig. 1c) fragment purified from the chicken oviduct and erythrocyte. The sequences were established as outlined in Fig. 1c and in the Methods section using the method of Maxam and Gilbert (41). In the upper figure single nucleotide differences between the two sequences are boxed and the nucleotide deletion/insertion sequence is indicated by vertical arrows. The lower part of the figure shows an autoradiogram of a sequencing gel. The sequence was read from the non-coding strand. To obtain this sequence the products of a HindIII digestion of the plasmid pBR(Eco6-Pst4)Ov and Er were [^{32}P]-labelled at their extremities. After digestion with the restriction enzyme EcoRI, the fragment HindIII-Eco6 (see Fig. 1c) was purified, subjected to chemical degradation and fractionated on denaturing 10 % polyacrylamide 8.3 M urea gels (41, 42). The sequence in the region of the insertion/deletion for the oviduct (Ov) and erythrocyte (Er) fragments are indicated.

from the non-coding strand were obtained by the technique of Maxam and Gilbert (41). The result of this experiment (see Fig. 6) shows that, in addition to two single base changes, there is a zone at 15 to 21 bp from the EcoRI site 6 in which there is a 4 bp insertion/deletion with two adjacent single base changes. To confirm this sequence we also sequenced the HindIII-Eco6 fragment on the coding strand by 5'-end labelling the EcoRI site followed by a secondary digestion at the HindIII site (results not shown).

DISCUSSION

Until recently, evidence for changes in the genomic arrangement of eukaryotic DNA has been obtained only when it has resulted in a difference which was sufficient to be detected by genetic experiments,by Cot analysis or by changes in the pattern of digestion by restriction enzymes. The existence of mechanisms for rearrangements of the DNA implied by these results (see introduction) raised the question as to the generality of the stability of DNA sequences from one tissue to another. In a first attempt to answer this question and to detect more subtle changes in the DNA sequence, we have analysed part of the chicken ovalbumin gene cloned from 2 different tissues using cosmids as vectors. The occurence of gross changes of greater than 50-110 bp was eliminated by restriction enzyme mapping and electron microscopic examination of heteroduplexes. A finer analysis was then performed using S1 nuclease to detect mismatches between the two sequences and finally we compared directly part of their DNA sequences.

For these experiments we optimized the conditions in which S1 nuclease was used to detect mismatches. Using a lower temperature, slightly higher NaCl concentration and non-denaturing gels (i.e. conditions under which S1 nuclease is less likely to digest mismatches than those used in this study) Shenk et al. (48) have claimed the detection of a mutation in SV40 which was believed, but not proven, to be due to a single-base change. Under their conditions we failed to detect a mismatch of 9 bp (F.G. unpublished results) that we had detected under our conditions (see methods section). Perhaps the difference in the

sensitivities is due to the source of enzyme used by Shenk et al. (48), or perhaps some of the assumptions on which they based their conclusion that they had detected a single-base change were ill-founded.

Our study has shown that we can detect with S1 nuclease a poorly hybridizing zone of 7 bp in a DNA:DNA heteroduplex. Consequently, we can conclude that there are no other such mismatches in the Eco6-Pst4 fragment analysed which includes the promoter region for the ovalbumin gene (23,47) and approximately 1300 bp 5' of this region (23).

It is difficult, and premature, to assert the significance of the insertion/deletion which we have detected. In view of the differences in the expression of the ovalbumin gene in the two tissues studied, a functional role could be suggested. This suggestion would be more plausible if the zone of difference was located in, or upstream from, the promoter region of the ovalbumin gene or at the intron-exon junctions where it might interfere with splicing of the ovalbumin RNA. However, the insertion/deletion occurs in the middle of intron A which argues against a functional significance as other studies (50, 51) have shown that the removal of large portions of introns or the introduction of additional sequences in an intron (52) has no apparent influence on the expression of genes. Another possibility is that minor changes in the DNA sequence are a common occurence at the somatic level and that further detailed analysis of corresponding DNA sequences from different tissues will confirm this variability. The change in the DNA sequence could also be at the germinal level and reflect the difference between the two chickens used for these studies. Previous studies have shown that there are 11 single nucleotide differences in a sequence of 2059 bp (exons and introns) cloned from the erythrocyte of two different chickens (53) and an analysis by restriction enzymes of the human globin genes (54) suggests that 1% of the sequences change from individual to individual. So we know that variations at a germinal level exist, but they are not as extensive as those which we describe in this paper. A finally possibility is that the differences that we detect are due to cloning artefacts. We recall that the DNA used in this study was originally cloned

using cosmids, a system of obvious value, but for which there is, as yet, not sufficient data available on its fidelity Many experiments have shown that very few mistakes occur when phage λ or plasmids are used as cloning vectors, though occassionally deletions can occur in these systems (for example see ref. 55). From our study we can nonetheless conclude that the region of the ovalbumin genome which is presumed to control transcription (namely the sequences 5' upstream from the leader exon) is the same in a tissue which transcribes Ov-mRNA frequently and in one in which transcription of Ov-mRNA cannot be detected. Therefore it seems unlikely that differences in the expression of this gene are due to minor tissue specific changes in the DNA sequences, though the more general significance, and role of these changes must be further studied.

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REFERENCES

1. Dunnick, W. (1979) Nature 281, 253-254
2. Perlman, S., Phillips, C. and Bishop, J.O. (1976) Cell 8, 33-42.
3. Alt, F.W., Kellems, R.E., Bertino, J.R. and Schimke, R.T. (1978) J. Biol. Chem. 253, 1357-1370.
4. Brown, D.D. and Dawid, I.B. (1968) Science 160, 272-280.
5. Gall, J.G. (1968) Proc. Natl. Acad. Sci. USA 60, 553-560.
6. Iwamura, Y., Sakai, M., Mita, T. and Muramatsu, M. (1979) Biochemistry 18, 5289-5294.
7. Spradling, A.C. and Mahowald, A.P. (1980) Proc. Natl. Acad. Sci. USA 77, 1096-1100.
8. Strom, C.M., Moscona, M. and Dorfman, A. (1978) Proc. Natl. Acad. Sci. USA 75, 4451-4454.
9. Wahl, G., Padgett, R.A. and Stark, G.A. (1979) J. Biol. Chem. 254, 8679-8689.
10. Leupold, U. (1980) Nature 283, 811-812.

11. Dickinson, D.G. and Baker, R.F. (1978) Proc. Natl. Acad. Sci. USA 75, 5627-5630.
12. McClintock, B. (1956) Cold Spring Harbor Symp. Quant. Biol. 21, 197-216.
13. Nisen, P., Medford, R., Mansour, J., Purucker, M., Skalka, A. and Shaprio, L. (1979) Proc. Natl. Acad. Sci. USA 76, 6240-6244.
14. Potter, S., Brorein, W.J. Jr., Dunsmuir, P. and Rubin, G.M. (1979) Cell 17, 415-428.
15. Zieg, J., Hilmen, M. and Simon, M. (1978) Cell 15, 237-244.
16. Silverman, M., Zieg, J., Hilman, M. and Simon, M. (1979) Proc. Natl. Acad. Sci. USA 76, 391-395.
17. Kamp, D., Kahmann, R., Zipser, E., Broker, T. and Chow, L. (1978) Nature 271, 577-580.
18. Rhoads, R.E., McKnight, G.S. and Schimke, R.T. (1971) J. Biol. Chem. 246, 7407-7410.
19. Bellard, M., Gannon, F. and Chambon, P. (1978) Cold Spring Harbor Symp. Quant. Biol. XLII, 779-791.
20. Palacios, R., Sullivan, D., Summers, N.M., Kiely, M.L. and Schimke, R.T. (1973) J. Biol. Chem. 248, 7530-7539.
21. Harris, S.E., Means, A.R., Mitchell, W.M. and O'Malley, B.W. (1973) Proc. Natl. Acad. Sci. USA 70, 3776-3780.
22. Palmiter, R.D. (1975) Cell 4, 189-197.
23. Gannon, F., O'Hare, K., Perrin, F., LePennec, J.P., Benoist, C., Cochet, M., Breathnach, R., Royal, A., Garapin, A., Cami, B. and Chambon, P. (1979) Nature 278, 428-434.
24. Mandel, J.L., Breathnach, R., Gerlinger, P., LeMeur, M., Gannon, F. and Chambon, P. (1978) Cell 14, 641-653.
25. Garapin, A.C., LePennec, J.P., Roskam, W., Perrin, F., Cami, B. Krust, A., Breathnach, R., Chambon, P. and Kourilsky, P. (1978) Nature 273, 349-354.
26. Mandel, J.L. and Chambon, P. (1979) Nucleic Acids Res. 7, 2081-2103.
27. LePennec, J.P., Baldacci, P., Perrin, F., Cami, B., Gerlinger, P., Krust, A., Kourilsky, P. and Chambon, P. (1978) Nucleic Acids Res. 5, 4547-4562.
28. Lai, E.C., Woo, S.L.C., Dugaiczyk, A. and O'Malley, B.W. (1979) Cell 16, 201-211.
29. Royal, A., Garapin, A., Cami, B., Perrin, F., Mandel, J.L., LeMeur, M., Brégégère, F., Gannon, F. LePennec, J.P., Chambon, P. and Kourilsky, P. (1979) Nature 279, 125-132.
30. Gross-Bellard, M., Oudet, P. and Chambon, P. (1973) Eur. J. Biochem. 36, 32-38.
31. Collins, J. and Brüning, H.J. (1978) Gene 4, 85-107.
32. Murray, N.E., Brammar, W.J. and Murray, K. (1976) Molec. Gen. Genet. 150, 53-61.
33. Hohn, B. (1979) Methods in Enzymology 68, 299-309.
34. Clewell, D.B. and Helinski, D.R. (1969) Proc. Natl. Acad. Sci. USA 62, 1159-1166.
35. McDonell, M.W., Simon, M.W. and Studier, F.W. (1977) J. Mol. Biol. 110, 119-146.
36. Breathnach, R., Mandel, J.L. and Chambon, P. (1977) Nature 270, 314-319.
37. Southern, E.M. (1975) J. Mol. Biol. 98, 503-518.
38. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
39. Sutcliffe J.G. (1978) Nucleic Acids Res. 5, 2721-2728.

40. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. and Chambon, P. (1978) Proc. Natl. Acad. Sci. USA 75, 4853-4857.
41. Maxam, A.M. and Gilbert, W. (1980) Methods in Enzymology 65, Part I, 499-560.
42. Sanger, F. and Coulson, A.R. (1977) FEBS Lett. 87, 107-110.
43. Sümegi, J., Breedveld, D., Hossenlopp, P., and Chambon, P. (1977) Biochem. Biophys. Res. Commun. 76, 78-85.
44. Humphries, P., Gordon, R.L., McConnel, D.J. and Connolly, P. (1974) Virology 58, 25-31.
45. Collins, J. (1979) Methods in Enzymology 68, 309-326.
46. Heilig, R., Perrin, F., Gannon, F., Mandel, J.L. and Chambon, P. (1980) Cell, in press.
47. Wasylyk, B., Kédinger, C., Corden, J., Brison, O. and Chambon, P. (1980) Nature 285, 367-373.
48. Shenk, T.E., Rhodes, C., Rigby, P.W.J. and Berg, P. (1975) Proc. Natl. Acad. Sci. USA 72, 989-993.
49. Fanning, T.G., Schreier, P.H., Büchel, D.E. and Davies, R.W. (1977) Analytical Biochemistry 81, 57-64.
50. Thimmappaya, B. and Shenk, T. (1979) J. Virol. 30, 668-673.
51. Volckaert, G., Feunteun, J., Crawford, L., Berg, P. and Fiers, W. (1979) J. Virol. 30, 674-682.
52. Johnson, J.D., Ogden, R., Johnson, P., Abelson, J., D mb ck, P. and Itakura, K. (1980) Proc. Natl. Acad. Sci. USA 77, 2564-2568.
53. Benoist, C., O'Hare, K., Breathnach, R. and Chambon, P. (1980) Nucleic Acids Res. 8, 127-142.
54. Jeffreys, A.J. (1979) Cell 18, 1-10.
55. Fritsch, E.F., Lawn, R.M. and Maniatis, T. (1980) Cell 19, 959-972.